

endo sequence from Collagen XVIII.

Sequence Range: 1-555

Nucleotide 1 = Start for Endostatin and fragments EM1 and EM2.

EM1 fragment ends at nucleotide 525, EM2 fragment ends at nucleotide 501.

5	10	15	20	25	30	35	40	45
CAT	ACT	CAT	CAG	GAC	TTT	CAG	CCA	GTG
GTA	TGA	GTA	GTC	CTG	AAA	GTC	GGT	CAC
50	55	60	65	70	75	80	85	90
ACC	CCC	CTG	TCT	GGA	GGC	ATG	CGT	GAT
TGG	GGG	GAC	AGA	CCT	CCG	TAC	GCA	GAC
100	105	110	115	120	125	130	135	140
TGC	TTC	CAG	CAA	GCC	CGA	GCC	GTG	GGG
ACG	AAG	GTC	GTT	CGG	GCT	CGG	CAC	CCC
145	150	155	160	165	170	175	180	185
TTC	CTG	TCC	TCT	AGG	CTG	CAG	GAT	ATC
AAG	GAC	AGG	AGA	TCC	GAC	GTC	CTA	TAC
195	200	205	210	215	220	225	230	235
GAC	CGG	GGG	TCT	GTG	CCC	ATC	GTC	AAC
CTG	GCC	CCC	AGA	CAC	GGG	TAG	CAG	GAC
245	250	255	260	265	270	275	280	285
CCC	AGC	TGG	GAC	TCC	CTG	TTT	TCT	GGC
GGG	TCG	ACC	CTG	AGG	GAC	AAA	AGA	CCG
290	295	300	305	310	315	320	325	330
GGG	GCC	CGC	ATC	TTT	TCT	TTT	GAC	GGC
CCC	CGG	GCG	TAG	AAA	AGA	AAA	CTG	CCA
340	345	350	355	360	365	370	375	380
GCC	TGG	CCG	CAG	AAG	AGC	GTA	TGG	CAC
CGG	ACC	GGC	GTC	TTC	TCG	CAT	ACC	GTG
385	390	395	400	405	410	415	420	425
AGG	CTG	ATG	GAG	AGT	TAC	TGT	GAG	ACA
TCC	GAC	TAC	CTC	TCA	ATG	ACA	CTC	TGT
435	440	445	450	455	460	465	470	475
GCT	ACA	GGT	CAG	GCC	TCC	TCC	CTG	GAA
CGA	TGT	CCA	GTC	CGG	AGG	AGG	GAC	AGT
485	490	495	500	505	510	515	520	525
AAA	GCT	GCG	AGC	TGC	CAC	AAC	AGC	TAC
TTT	CGA	CGC	TCG	ACG	GTG	TTG	TCG	ATG
530	535	540	545	550	555			
AGC	TTC	ATG	ACC	TCT	TTC	TCC	AAA	TAG
TCG	AAG	TAC	TGG	AGA	AAG	AGG	TTT	ATC

Sequence Range: 1 to 184

5	10	15	20	25	30	35	40	45						
HTH	QDF	QPV	LHL	VAL	NTP	LSG	GMR	GIR	GAD	FQC	FQQ	ARA	VGL	SGT
50	55	60	65	70	75	80	85	90						
FRA	FLS	SRL	QDL	YSI	VRR	ADR	GSV	PIV	NLK	DEV	LSP	SWD	SLF	SGS
95	100	105	110	115	120	125	130	135						
QGQ	LQP	GAR	IFS	FDG	RDV	LRH	PAW	PQK	SVW	HGS	DPS	GRR	LME	SYC
140	145	150	155	160	165	170	175	180						
ETW	RTE	TTG	ATG	QAS	SLL	SGR	LLE	QKA	ASC	HNS	YIV	LCI	ENS	FMT
SFS K														

Fig. 2

1440.1023-011

FIG. 3

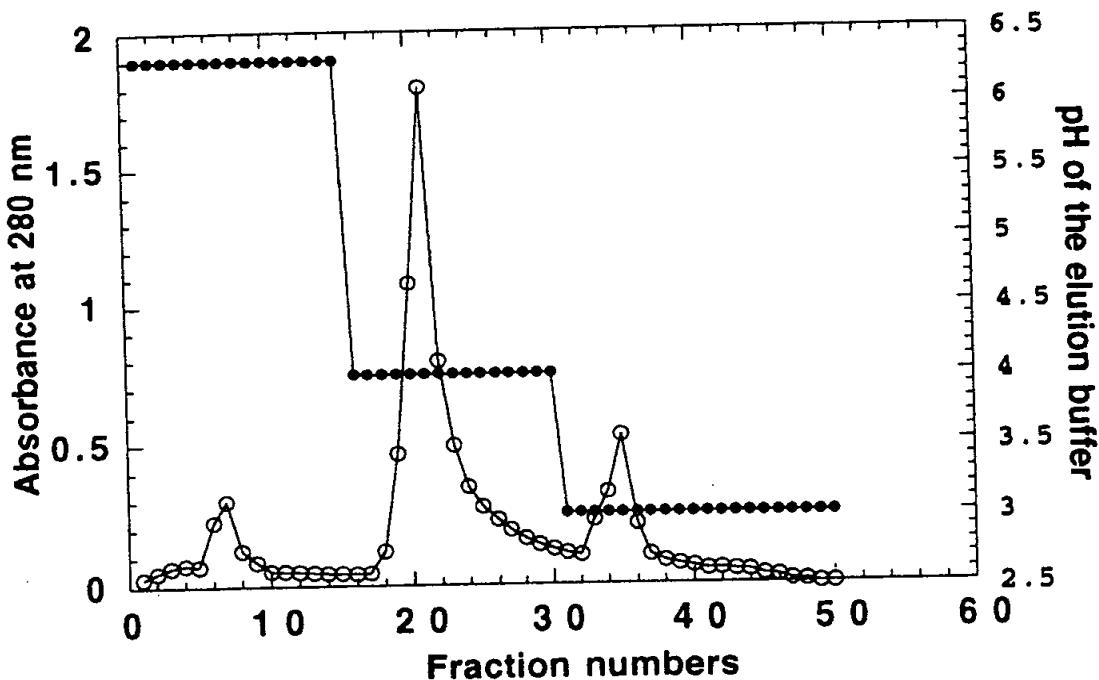
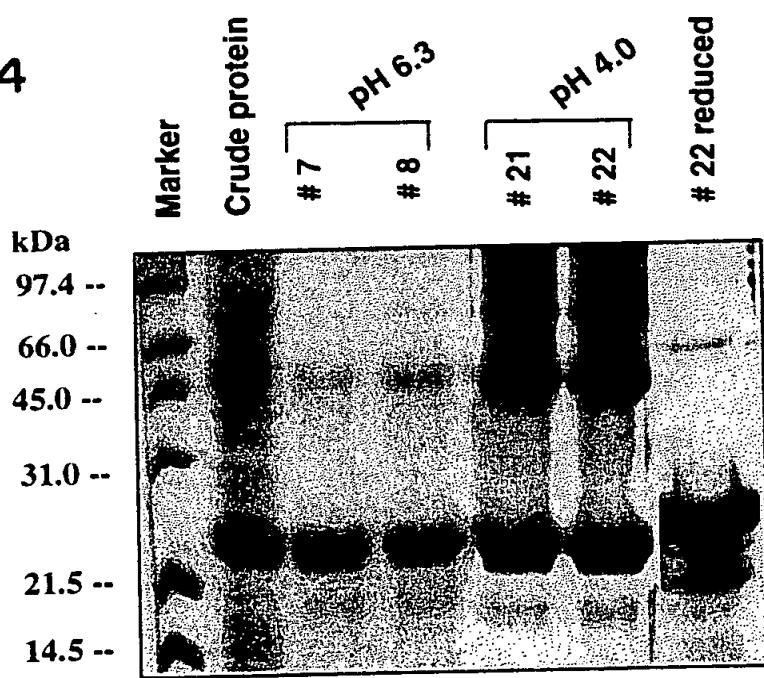


FIG. 4



1440.1023-011

FIG.5

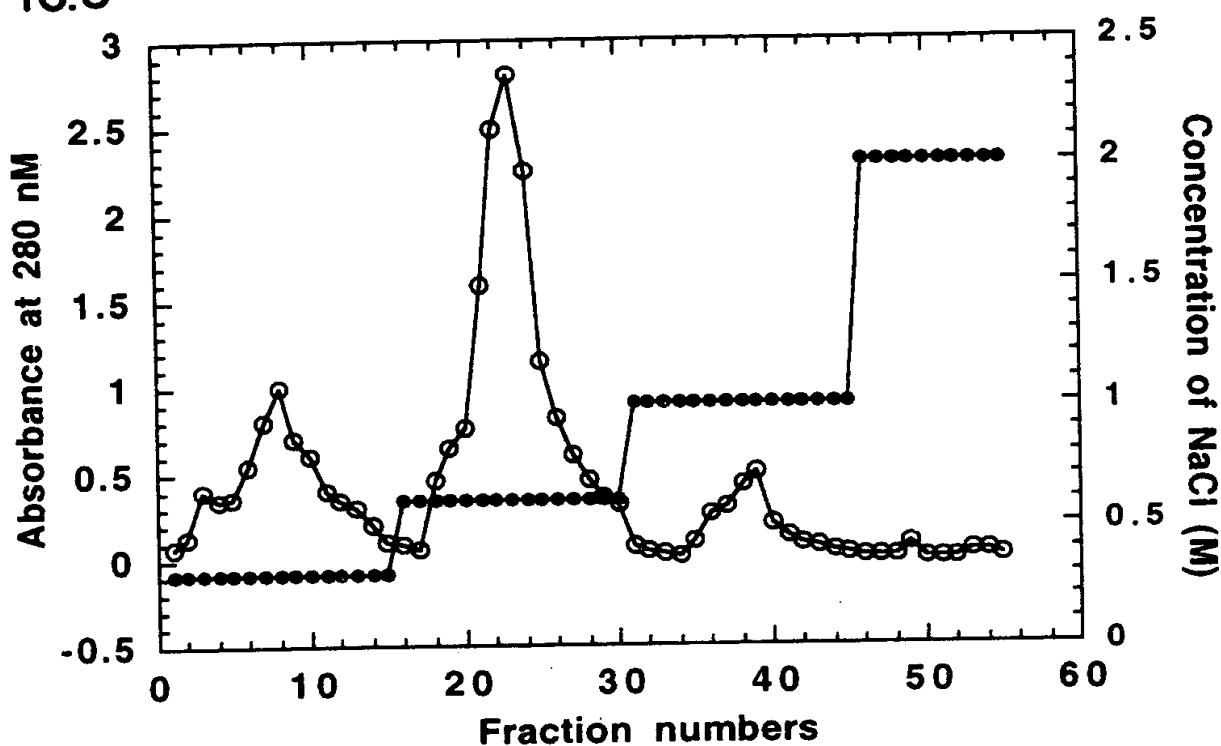
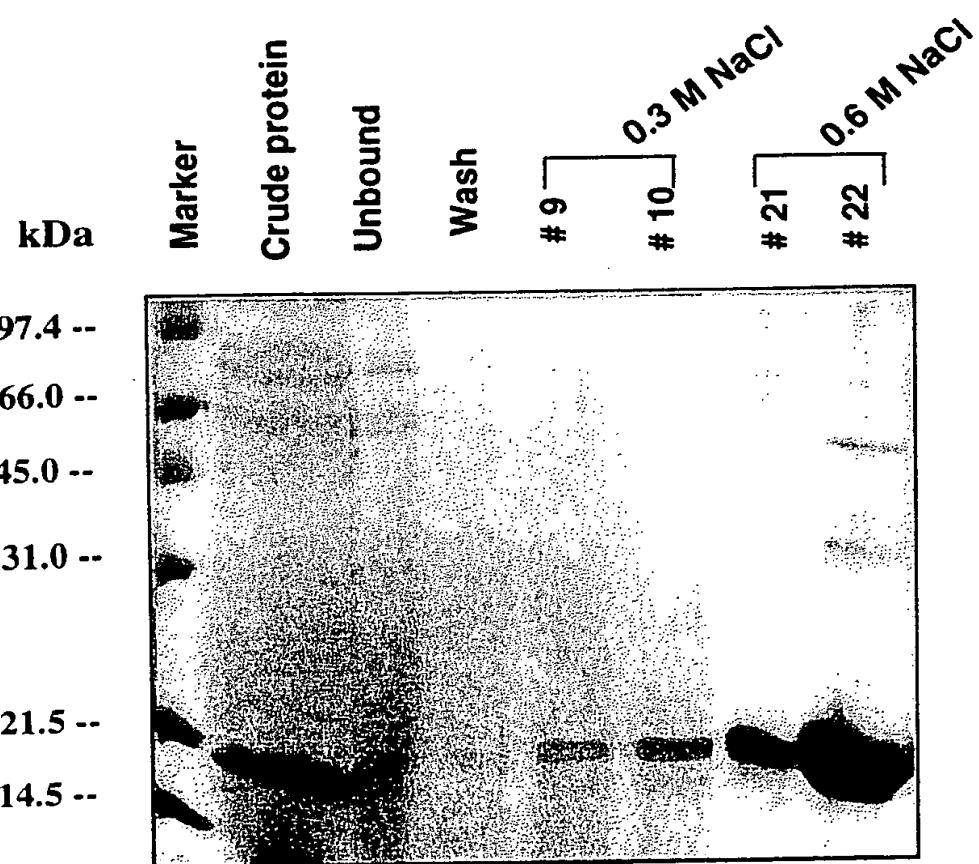


FIG.6



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FIG.7

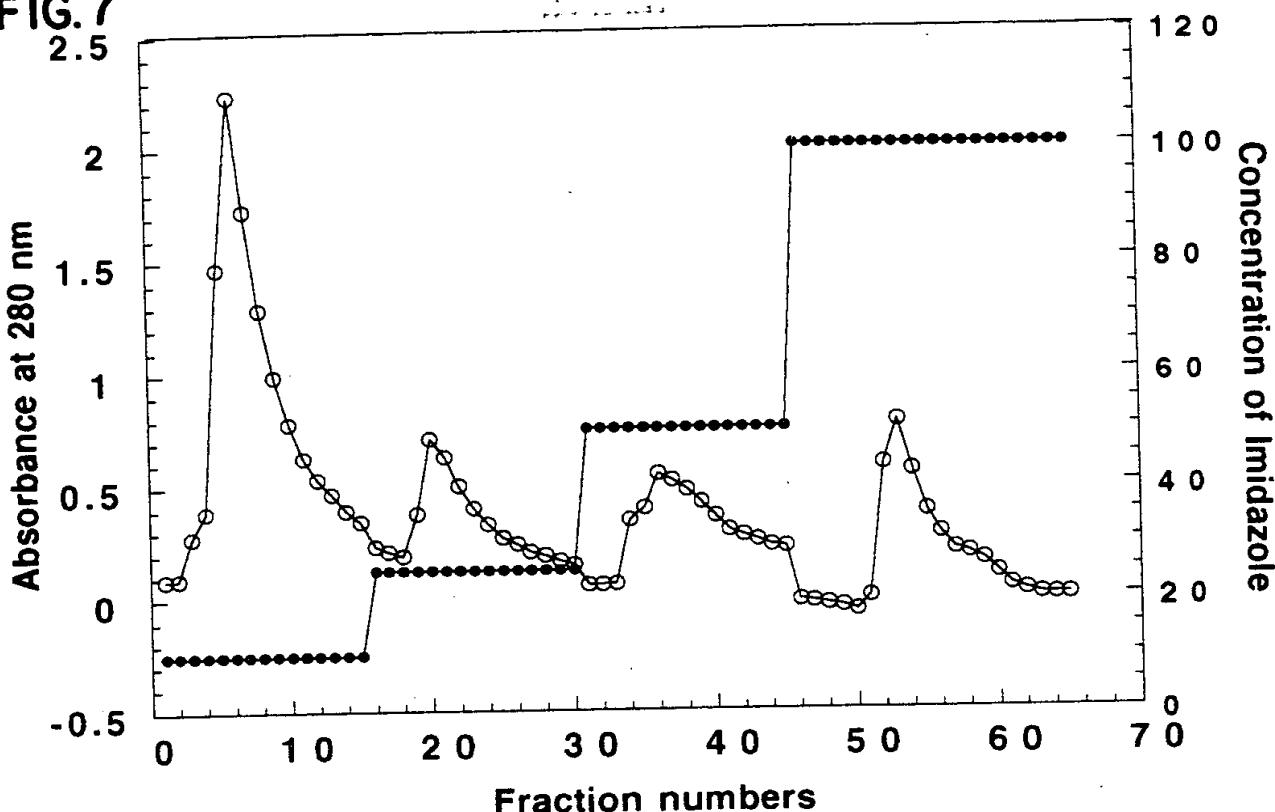
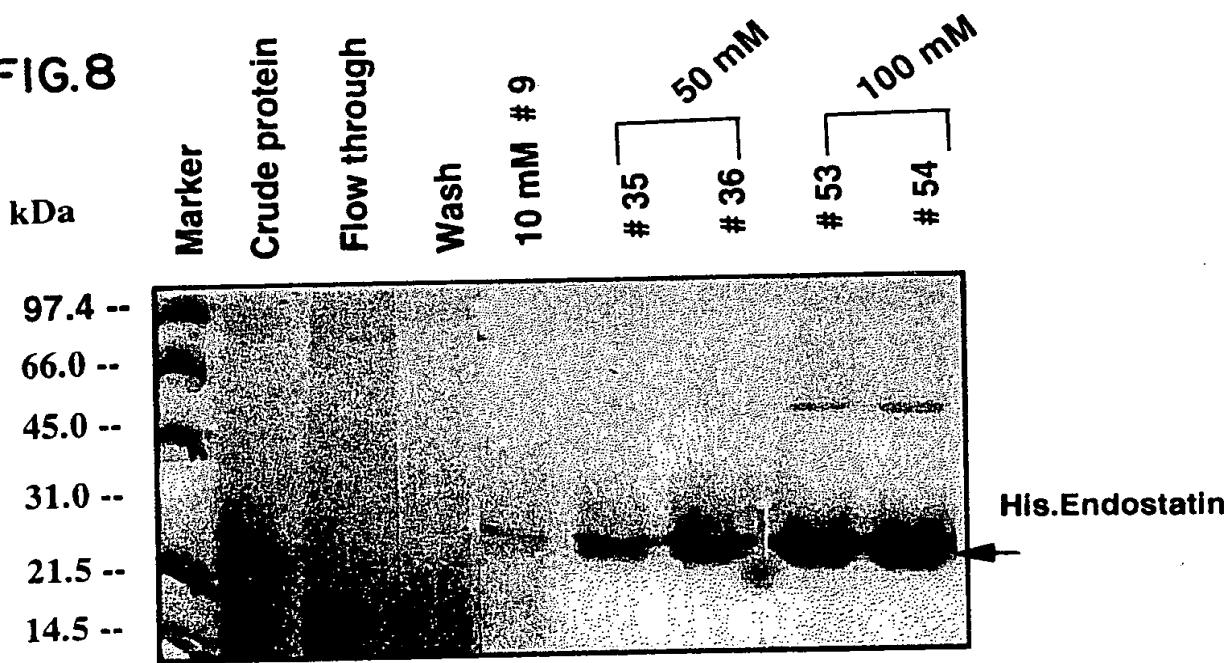


FIG.8



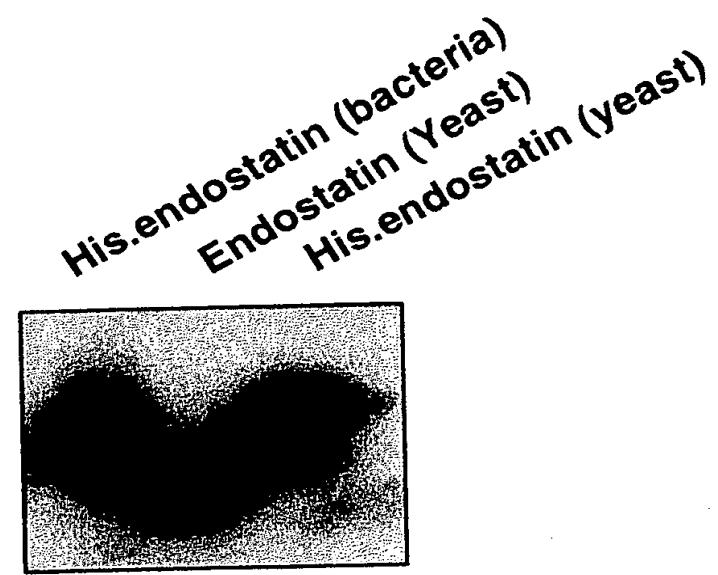
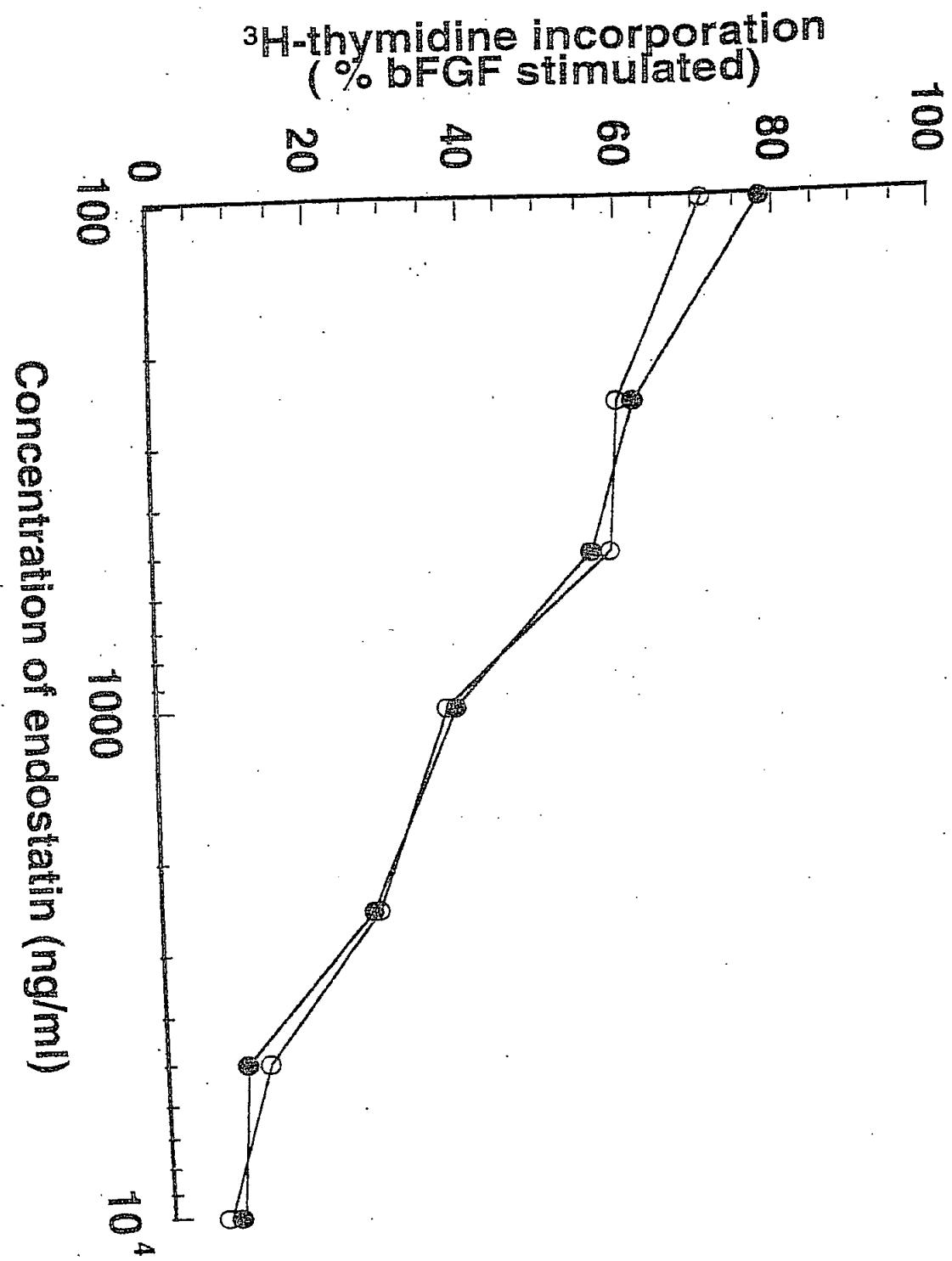
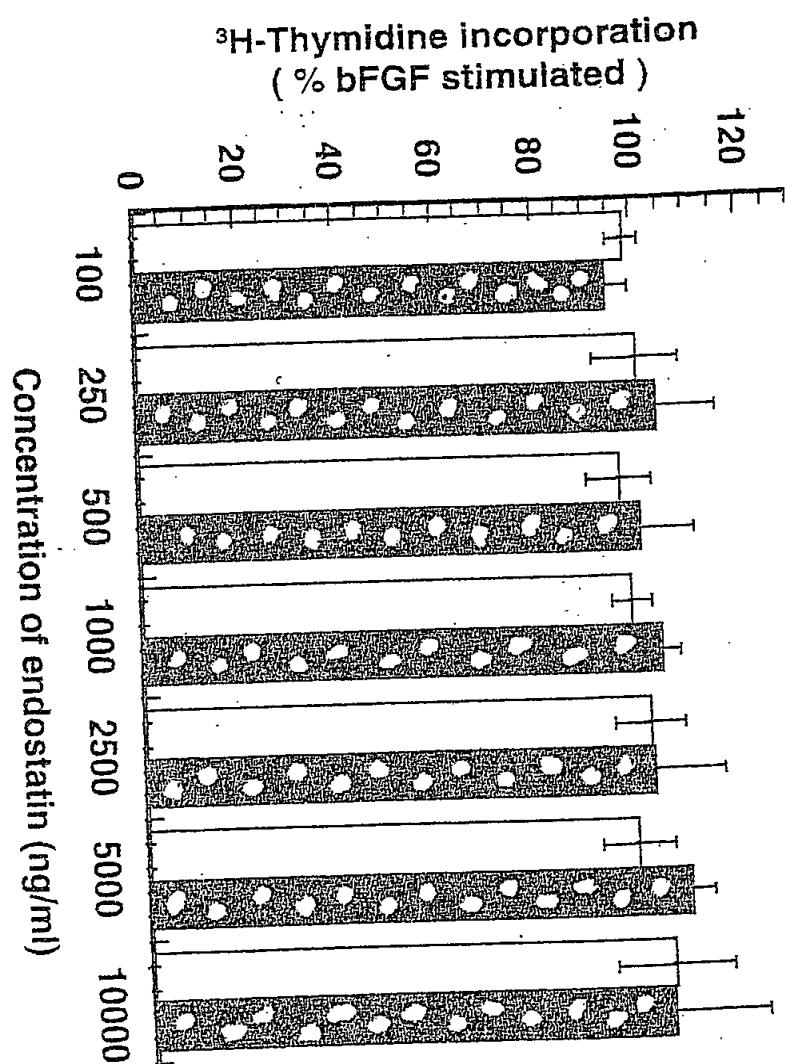
**FIG. 9**

Fig. 10



1440.1023-11

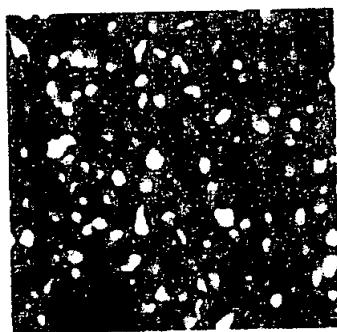
Fig. 11



1440.1623-011

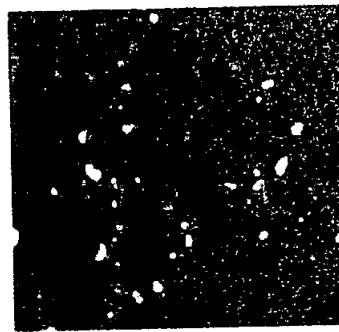
1440. 1023-011

FIG. I2 A



Control +bFGF

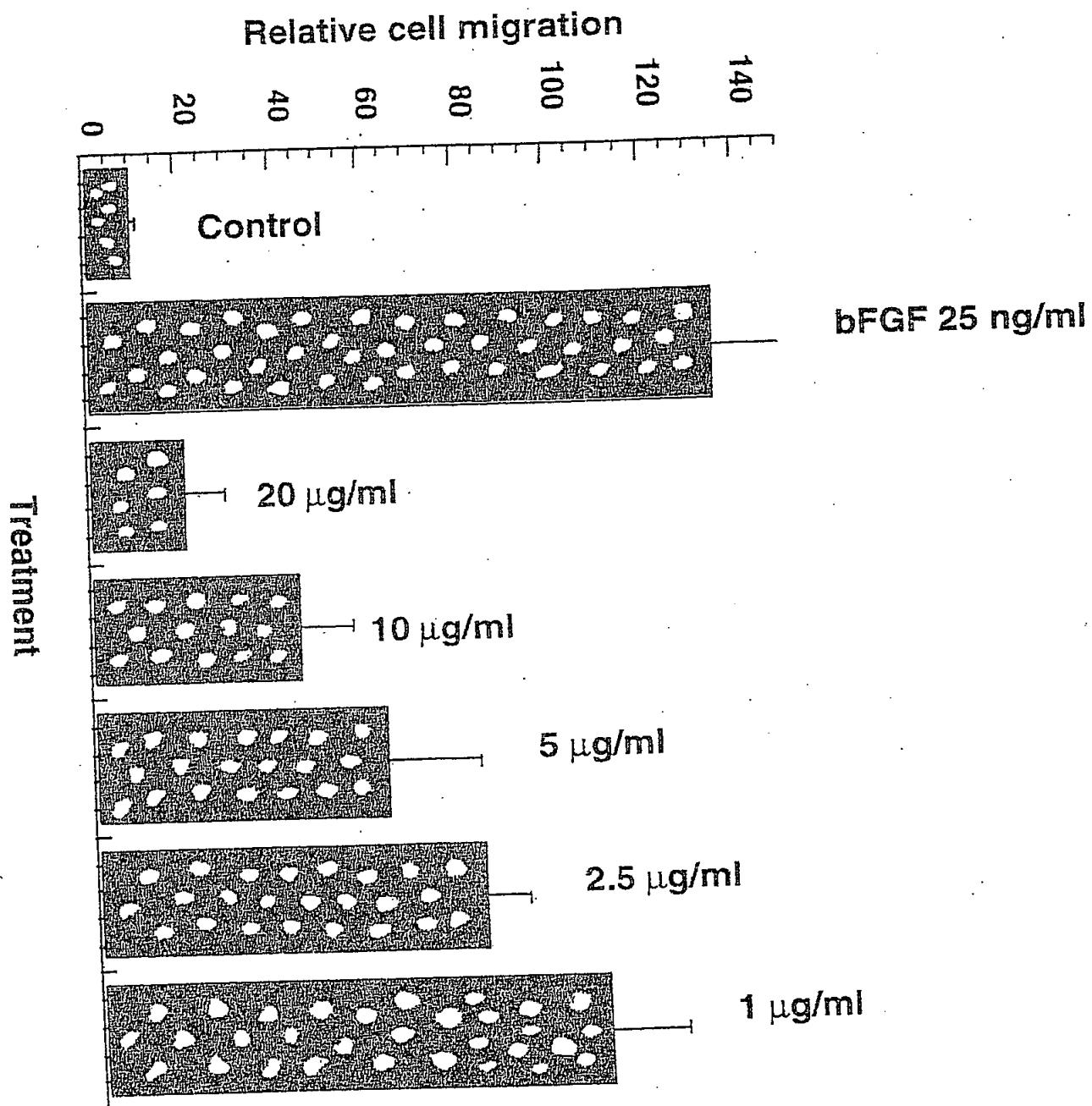
FIG.I2B



Endostatin 20  $\mu$ g/ml

0000000000 0000000000

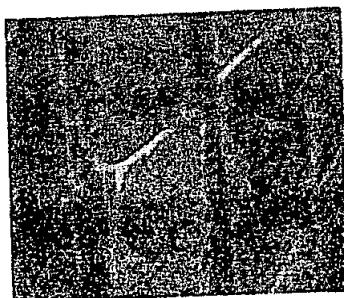
Fig. 13



1440.1023-A11

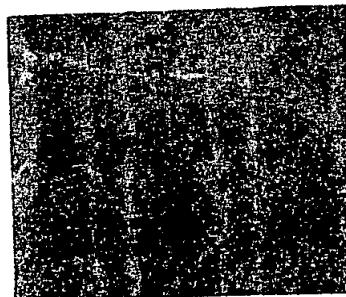
1440.1023-011

**FIG.14a**



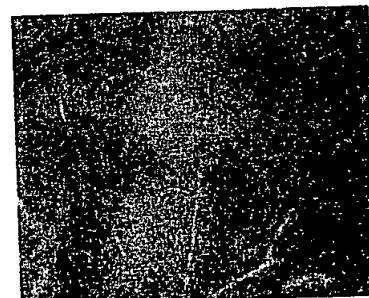
**Negative control**

**FIG.14b**



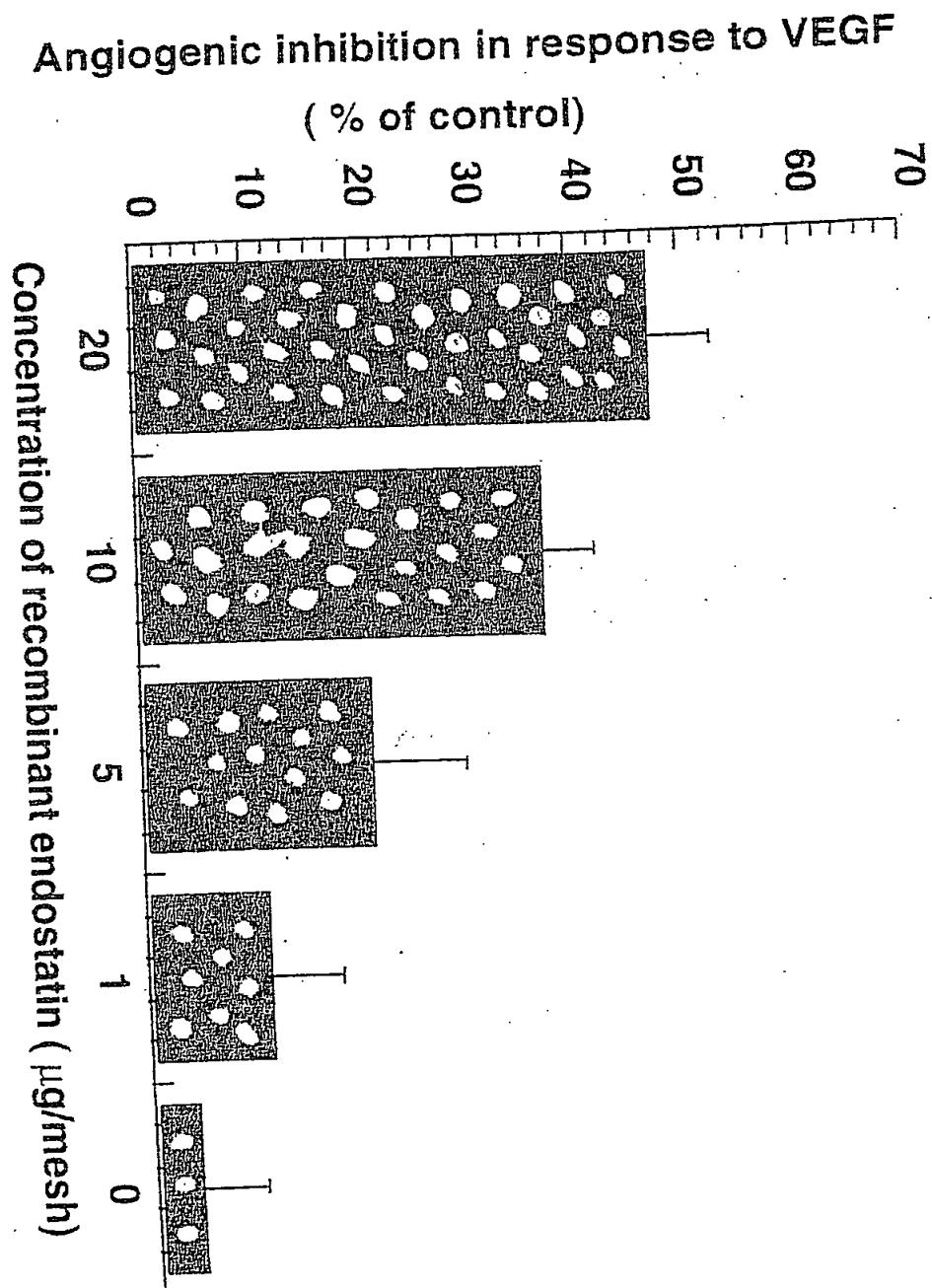
**Positive control  
(VEGF)**

**FIG.14c**



**VEGF +  
endostatin 20 µg**

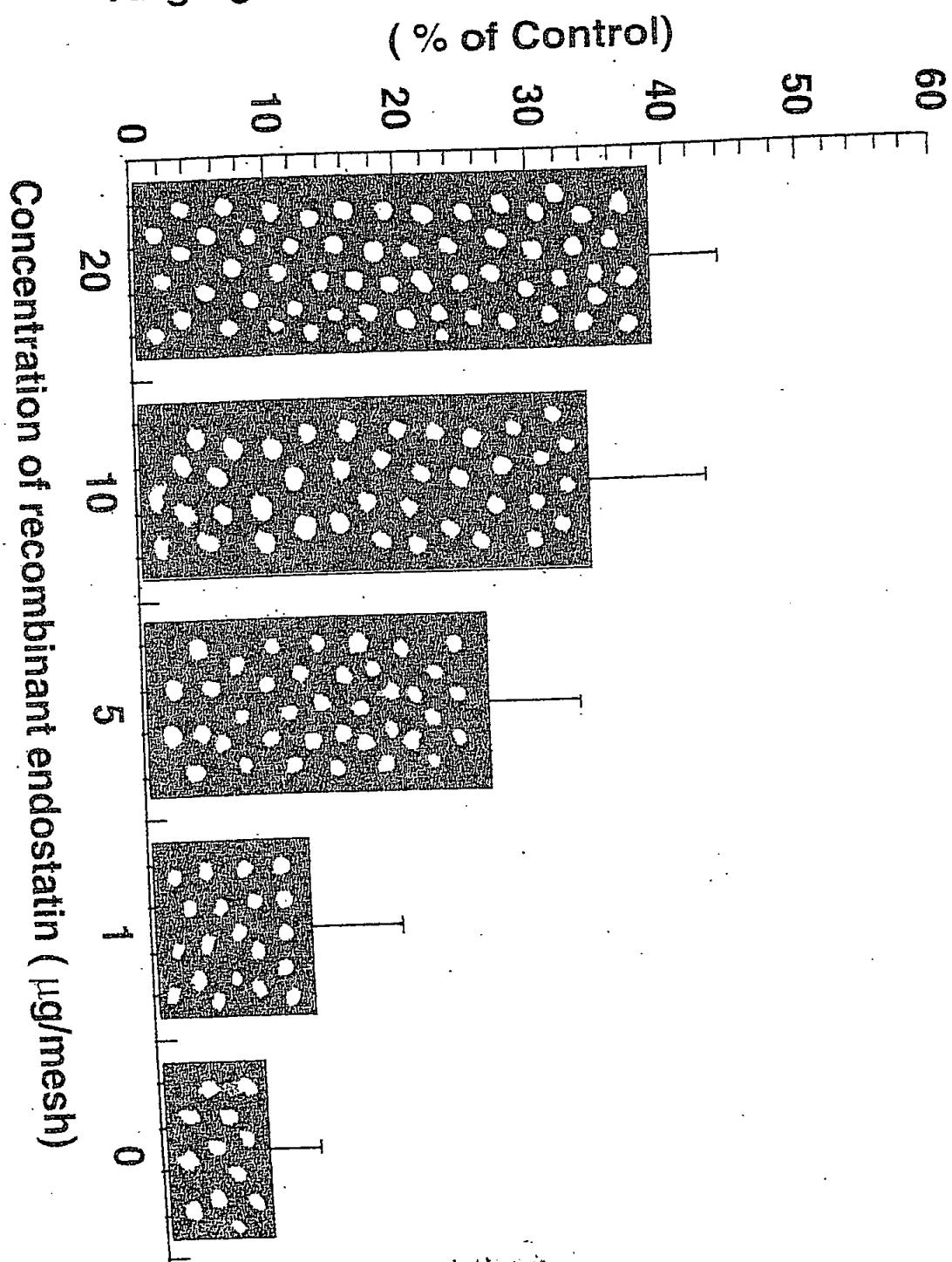
Fig. 15A



1440.1023-011

Fig. 15B

### Angiogenic inhibition in response to bFGF



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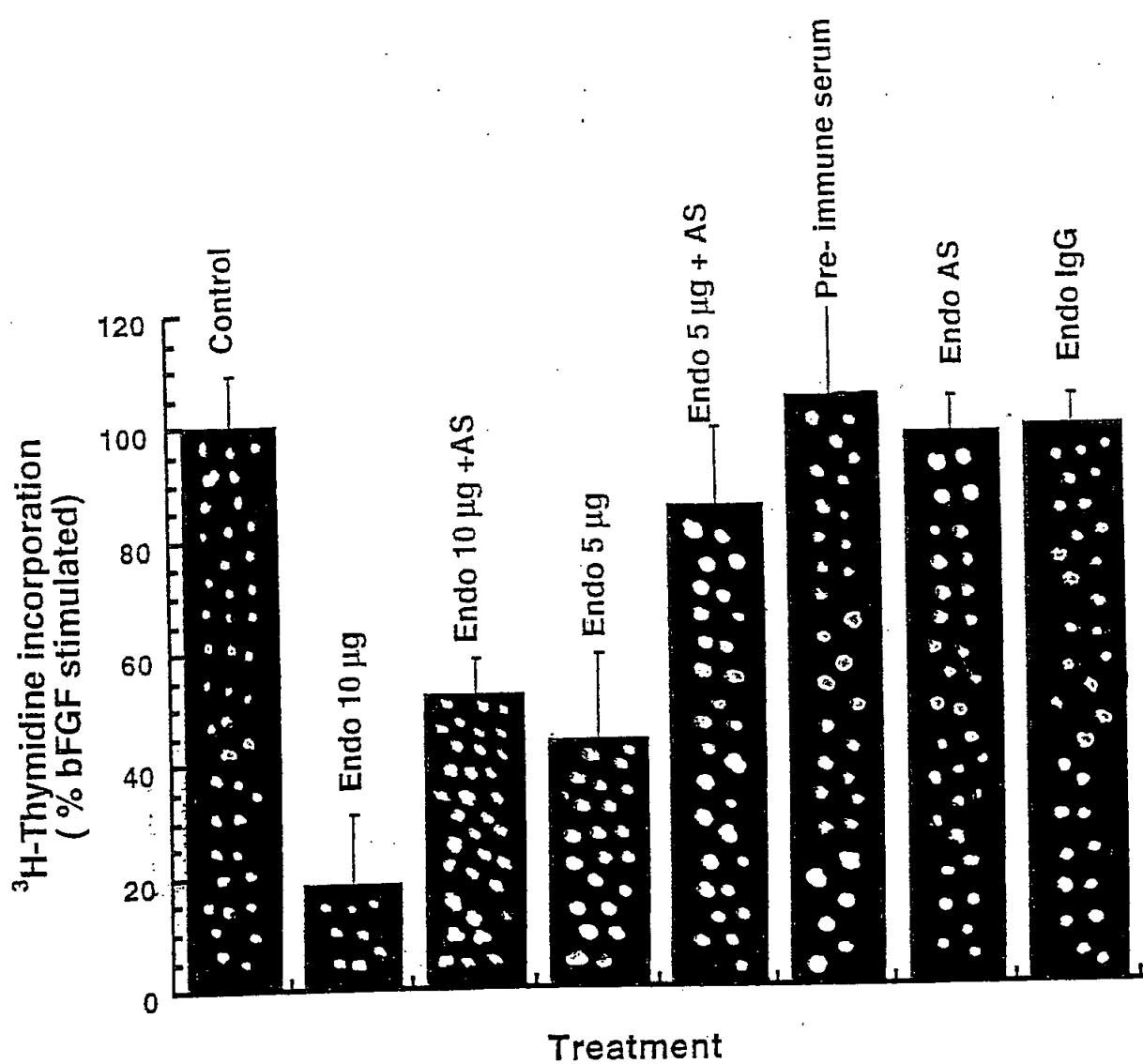
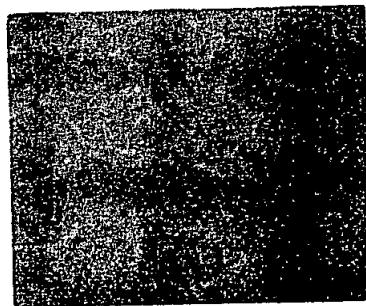


Fig. 16

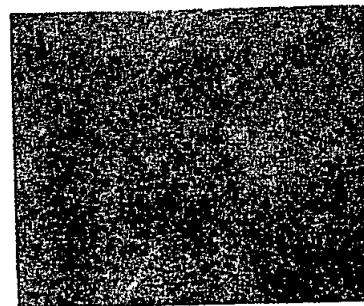
1440.1023-011

FIG.17 a



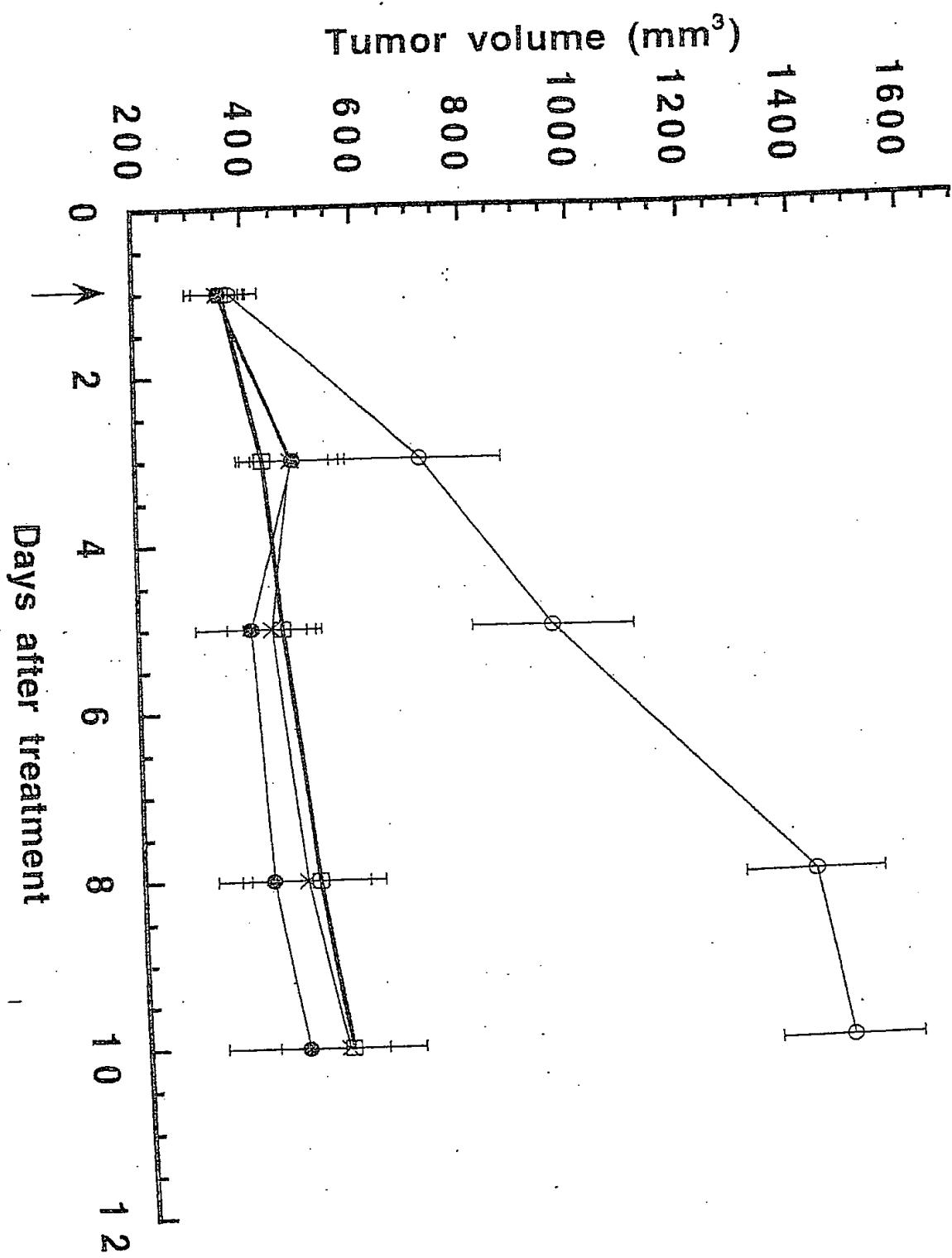
VEGF + endostatin (10 µg)

FIG.17 b



VEGF + endostatin (10 µg) +  
polyclonal antiserum (50 µg)

Fig. 18



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FIG.19A

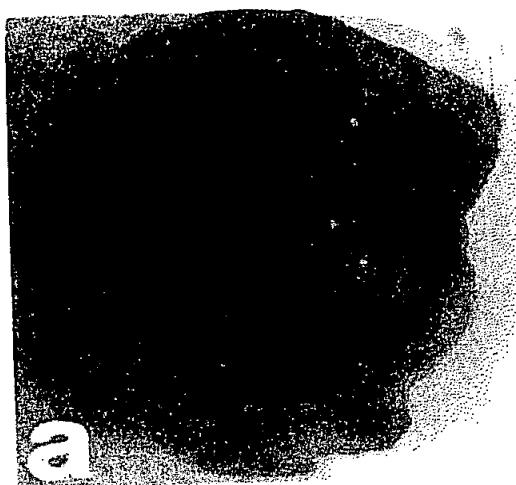


FIG.19B

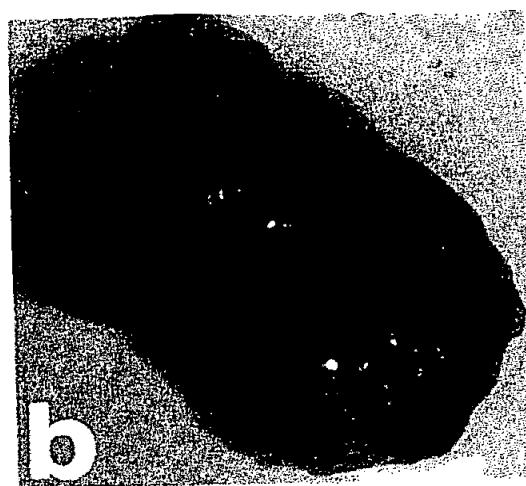


FIG.19C

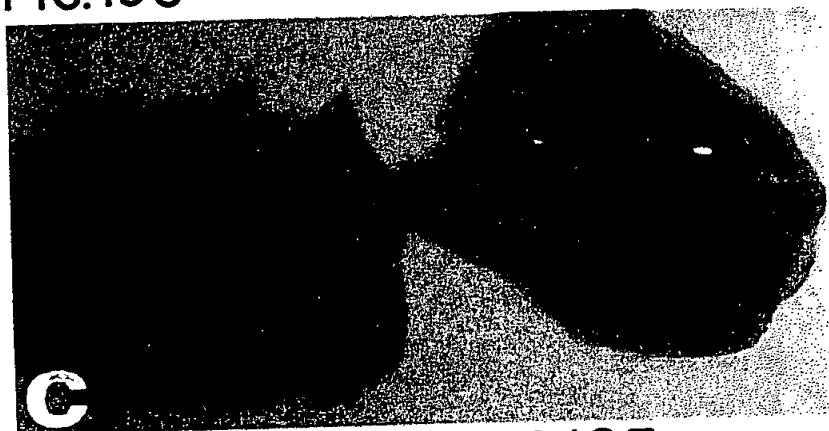


FIG.19D

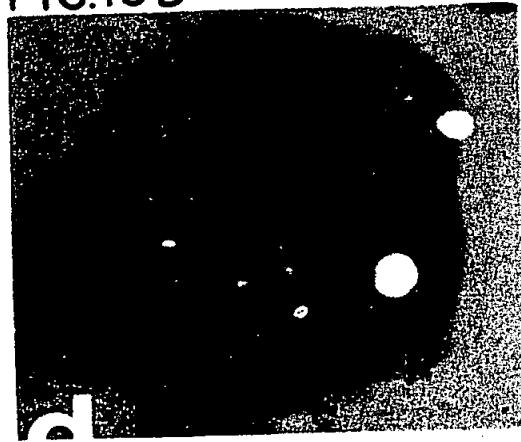
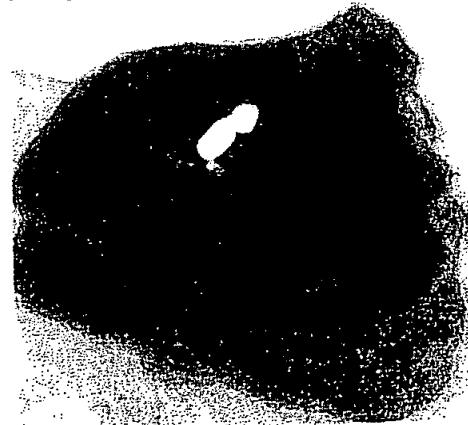
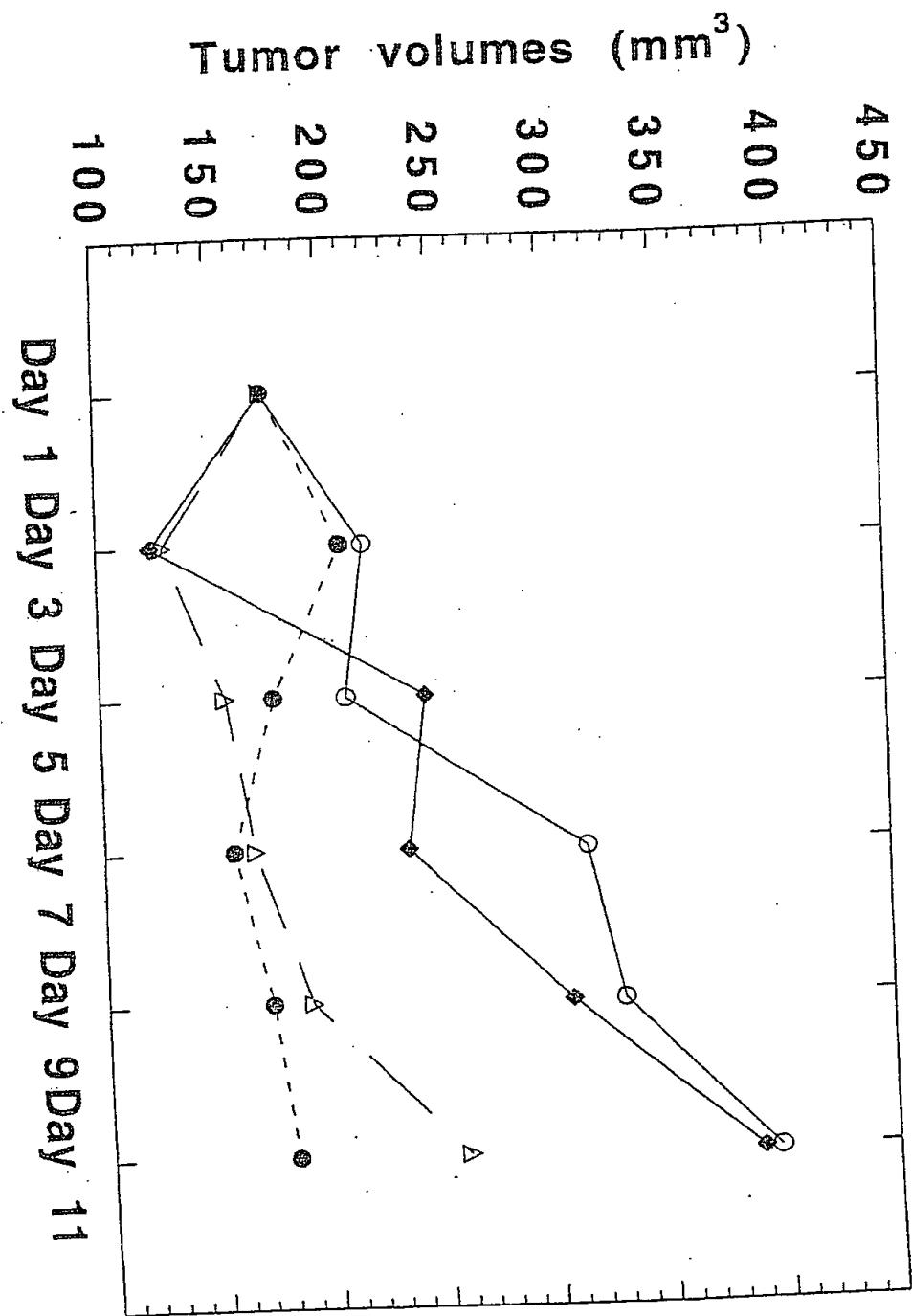


FIG.19E



00000000000000000000000000000000

Fig. 20



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Fig. 21

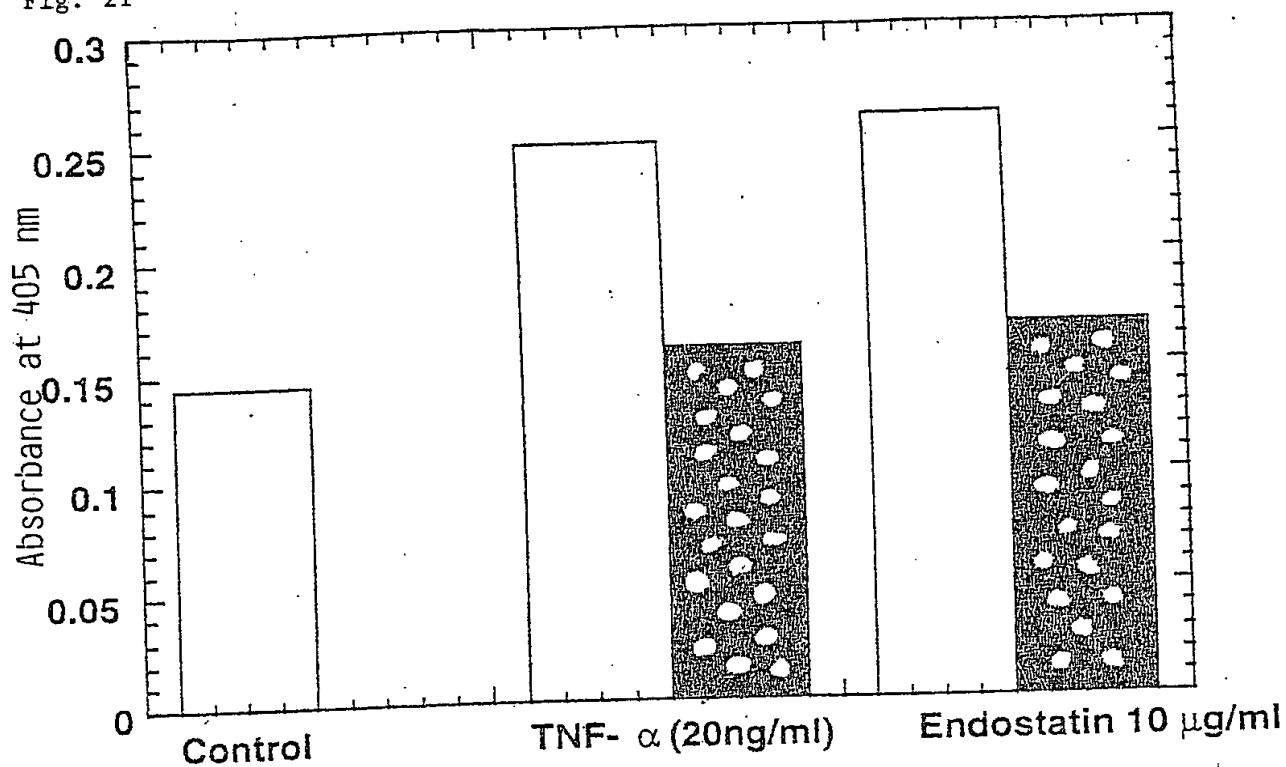


Fig. 22

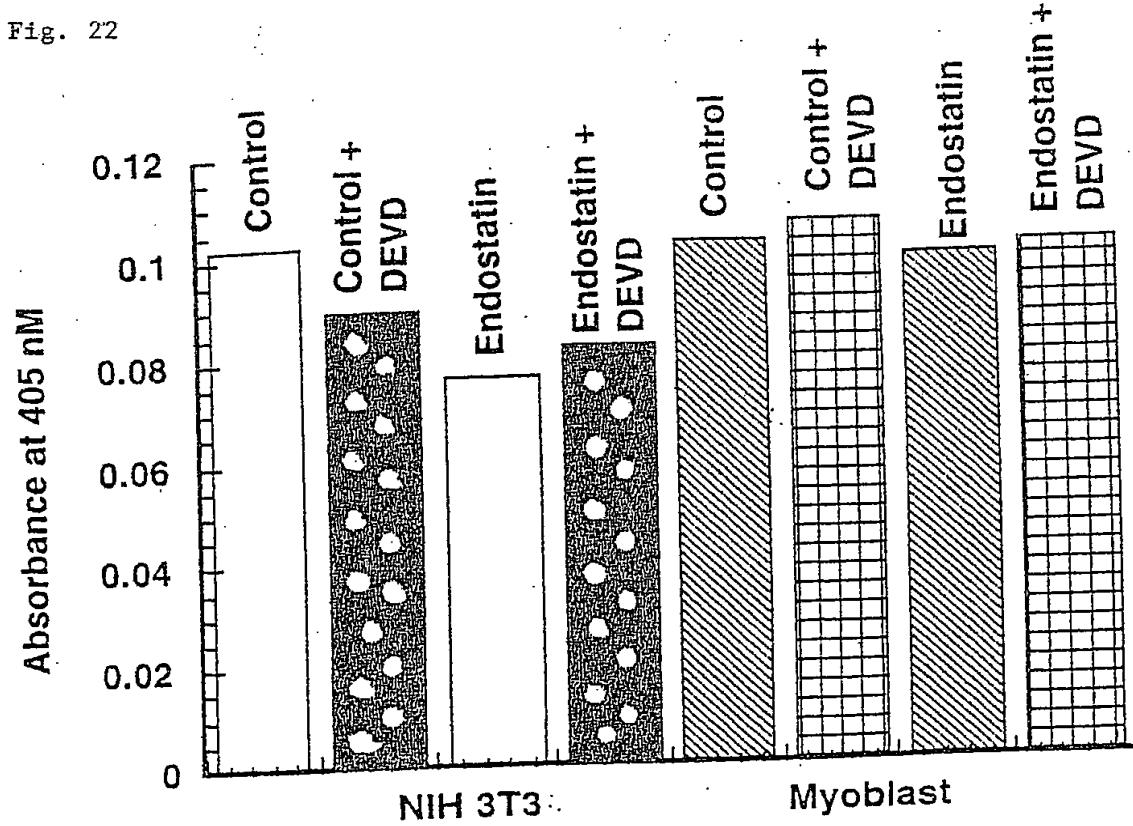
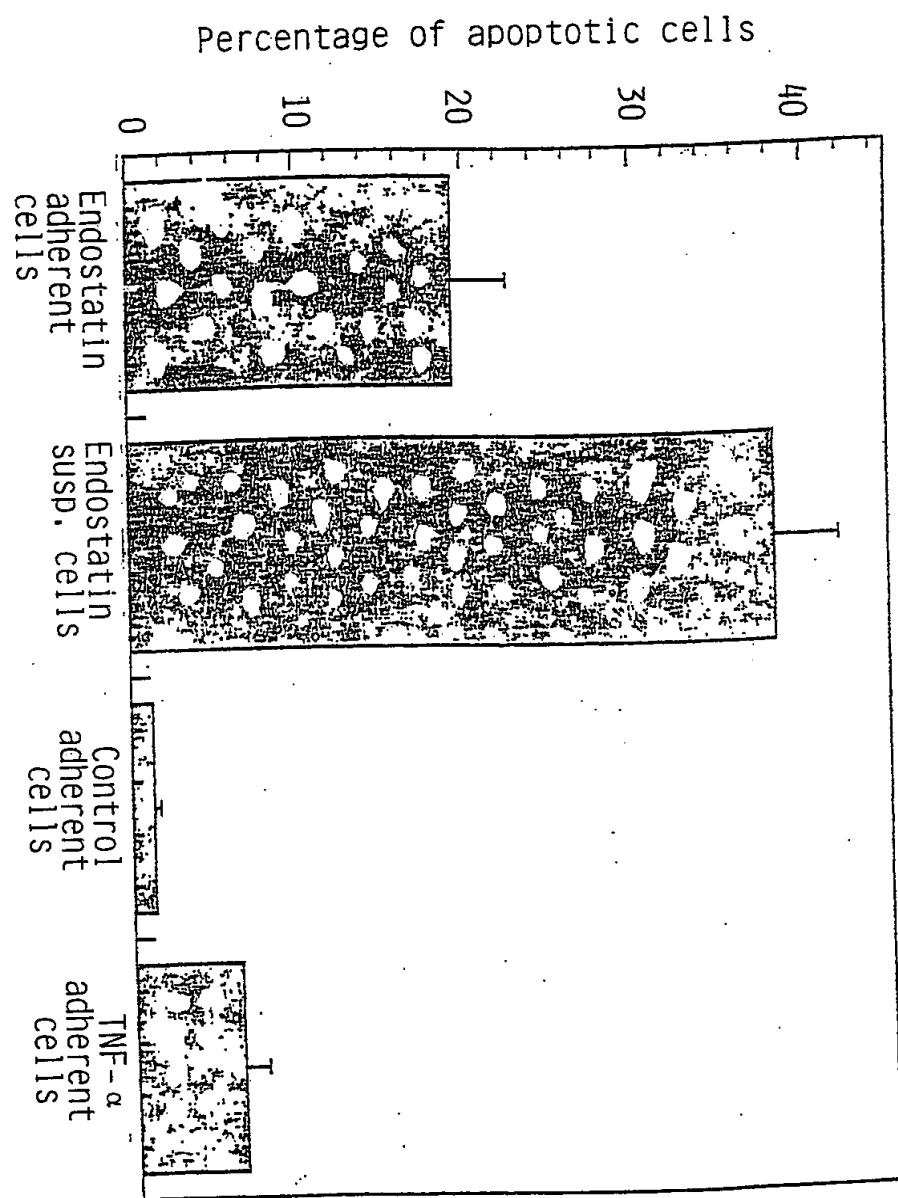


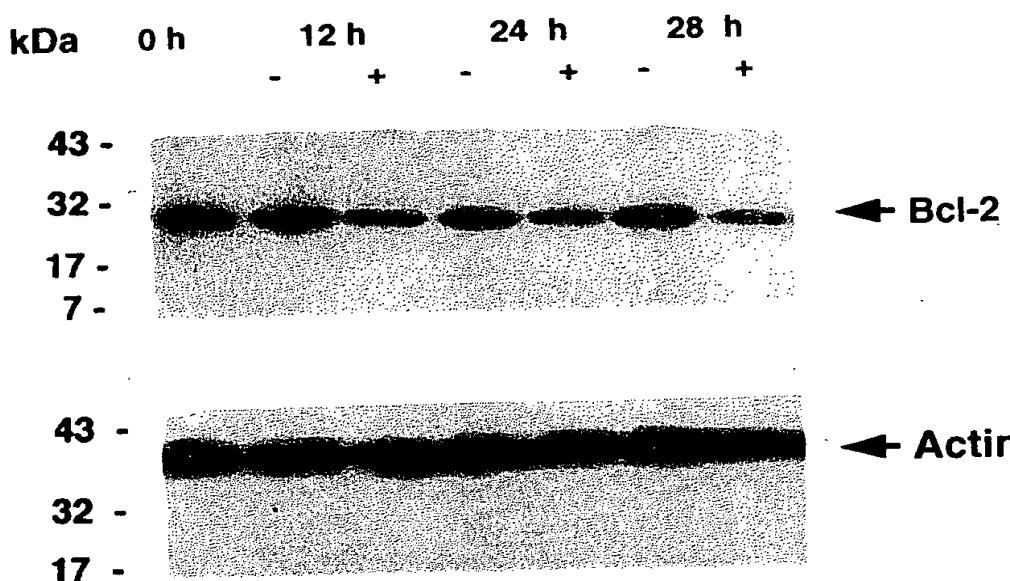
Fig. 23



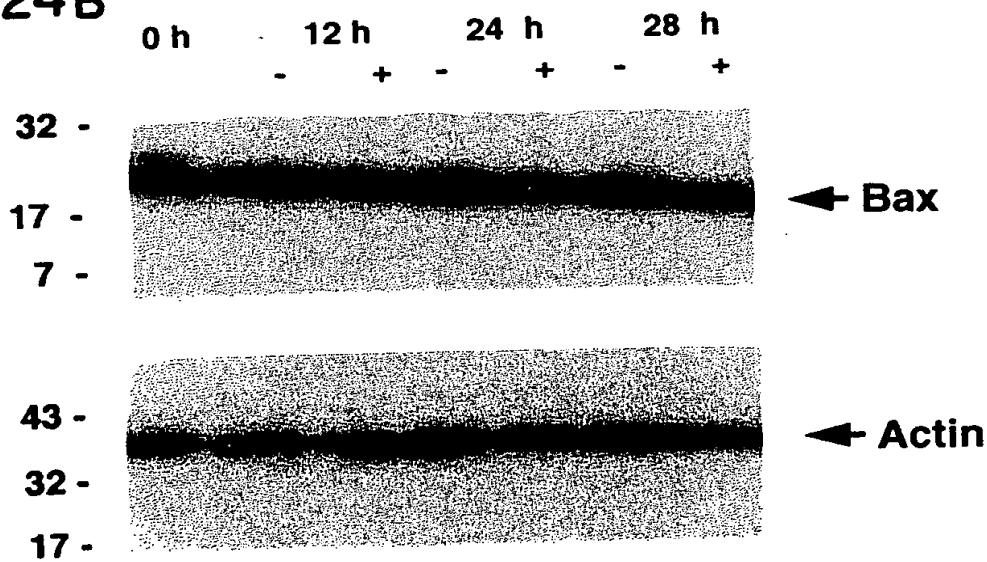
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1440.1023-011

**FIG. 24A**



**FIG. 24B**



1440.1023-011

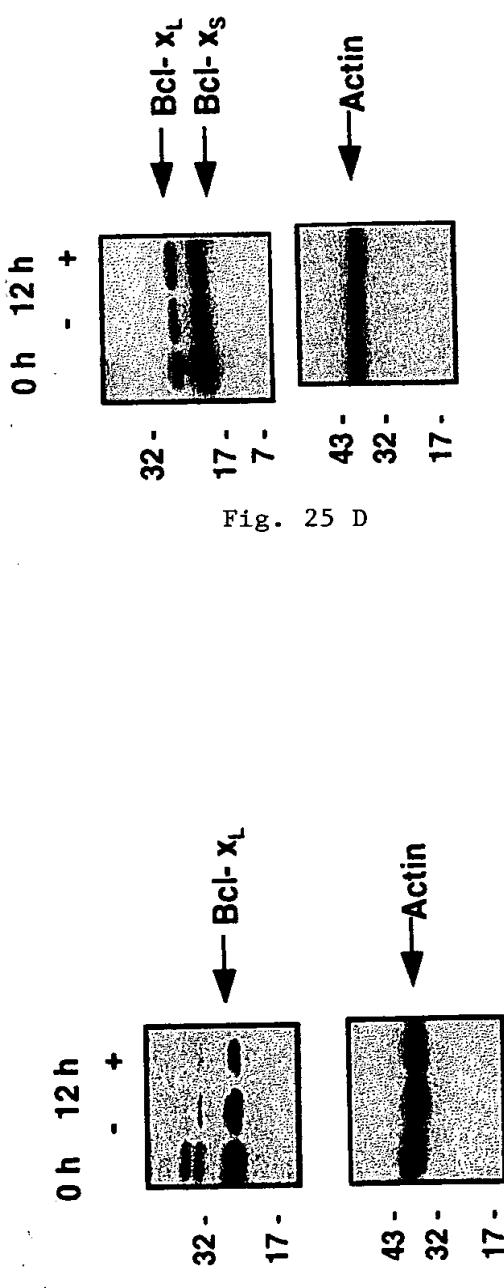
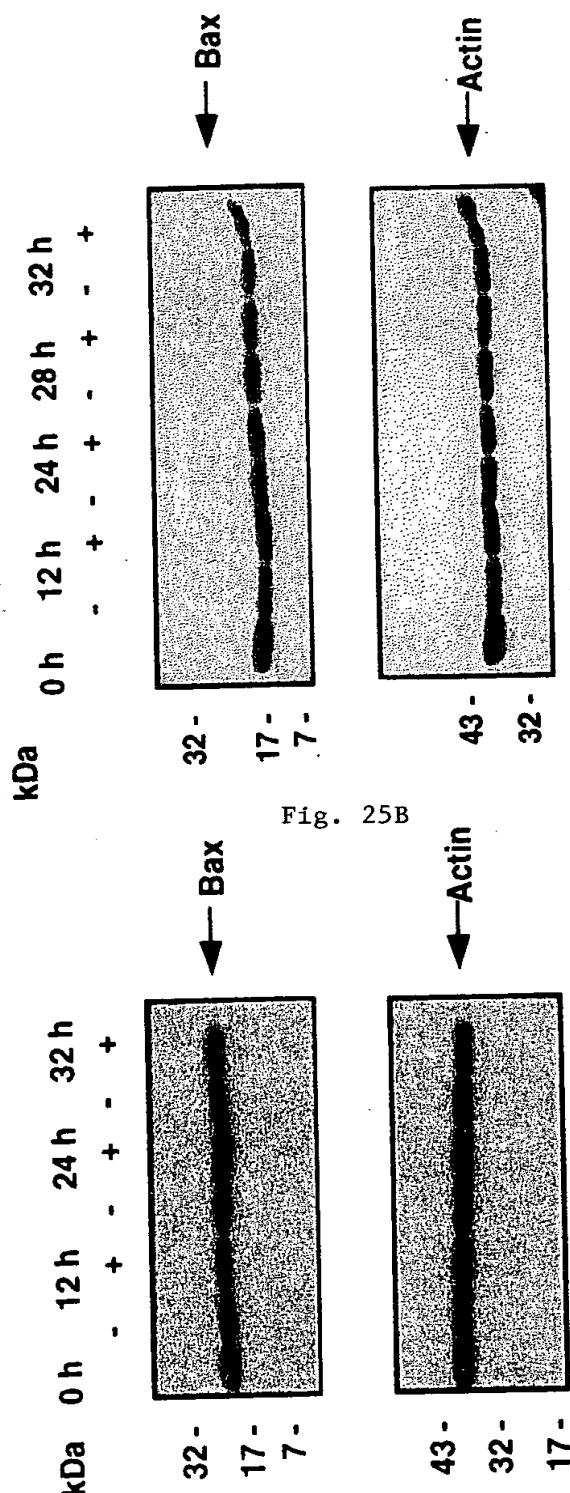


Fig. 25A

Fig. 25 D

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Construct Name	Primer Sequence	Cloning Sites	Vector	Protein Sequence
pET17b/ his.mendo	5'-GGC ATA TGC ATA CTC ATC AGG- ACT TT-3' (up) (SEQ ID NO:3)	<i>Nde</i> I & <i>Xba</i> I	Prokaryotic expression, pET	MGHHHHHHHHSSGHDDDKH M-mendo (SEQ ID NO:5)
	5' AAC TCG AGC TAT TTG GAG AAA- GAG GT-3' (down) (SEQ ID NO:4)			
pET28a/ mendo	5'-GGC ATA TGC ATA CTC ATC AGG- ACT TT-3' (up) (SEQ ID NO:3)	<i>Nde</i> I & <i>Not</i> I	Prokaryotic expression, pET	MGSSHHHHHHSSGLVPRGSHM- mendo (SEQ ID NO:7)
	5'-AAG CGG CCG CCT ATT TGG AGA- AAG AGG T-3' (down) (SEQ ID NO:6)			
pET28a/ EM-1	5' TTC CAT ATG CAT ACT CAT CAG- GAC TTT CAG CCA-3' (up) (SEQ ID NO:8)		Prokaryotic expression, pET	MGSSHHHHHHSSGLVPRGSHM-me ndo (SEQ ID NO:7)
	5' TTA GCG GCC GCCTAC TCA ATG- CAC AGG ACG ATG TA-3' (down) (SEQ ID NO:9)			
pET28a/ EM-2	5' TTC CAT ATG CAT ACT CAT CAG- GAC TTT CAG CCA-3' (up) (SEQ ID NO:8)		Prokaryotic expression, pET	MGSSHHHHHHSSGLVPRGSHM-me ndo (SEQ ID NO:7)
	5' TTA GCG GCC GCCTAG TTG TGG- CAG CTC GCA GCT TTC TG-3' (down) (SEQ ID NO:10)			

Fig. 26A

1440.1023-011

Construct Name	Primer Sequence	Cloning Sites	Vector	Protein Sequence
pPICZ $\alpha$ A/ mendo	5' GGG AAT TCC ATA CTC ATC ACG- ACT TT-3' (up) (SEQ ID NO:11)  5' AAG CGG CCT ATT TGG AGA- AAG AGG T-3' (down) (SEQ ID NO:6)	EcoRI & NotI	Eukaryotic expression, yeast/pPICZ $\alpha$ A	EF-mendo
pPICZ $\alpha$ A/ His.mendo	5' AAG AAT TCC ATC ATC ATC ATC- ATC ACA GCA GC-3' (up) (SEQ ID NO:12)  5' AAG CGG CCT ATT TGG AGA- AAG AGG T-3' (down) (SEQ ID NO:6)	EcoRI & NotI	Eukaryotic expression, yeast/pPICZ $\alpha$ A	KHM-mendo (SEQ ID NO:13)
pPICZ $\alpha$ A/ Hendo	5' TTT GAA TTC GCC CAC AGC CAC- CGC GAC TTC CAG CCC GTG CTC- CA-3' (up) (SEQ ID NO:14)	EcoRI & NotI	Eukaryotic expression, yeast/pPICZ $\alpha$ A	EF-hendo
	5' AAA AGC GGC CGC CTA CTT GGA- GGC AGT CAT GAA GCT GTT CTC- AA-3' (down) (SEQ ID NO:15)			
pPICZ $\alpha$ A/ Restin	5' TTT TTT GAA TTC ATT TCA AGT- GCC AAT TAT GAG AAG CCT GCT CTG CAT TTG-3' (up) (SEQ ID NO:16)  5' AAG AAT GCG GCG GCT TAC TTC- CTA GCG TCT GTC ATG AAA CTG- TTT TCG AT-3' (down) (SEQ ID NO:17)	EcoRI & NotI	Eukaryotic (Yeast), Pichia, pPICZ $\alpha$ A	EF-restin

Fig. 26B

1440. 1023-011

Construct Name	Primer Sequence	Cloning Sites	Vector	Protein Sequence
pPICZ $\alpha$ A/ His.Resin	5' AAT TCC ATC ACC ACC ATC ATC- ACG- 3' (up) (SEQ ID NO:18)	<i>Eco</i> RI (oligo insertion)	Eukaryotic (Yeast), Pichia, pPICZ $\alpha$ A	EFHHHHHHH-resin (SEQ ID NO:20)
	5' AAT TCG TGA TGG TGA TGG TGA- TGG-3' (down) (SEQ ID NO:19)			
pET28a/ apomigren	5' TTC CAT ATG ATA TAC TCC TTT- GAT GGT CGA GAC ATA ATG ACA-3' (up) (SEQ ID NO:21)	<i>Nde</i> I & <i>Not</i> I	Prokaryotic, pET system	MGSSHHHHHHSSGL VPRGSHM-apo migren (SEQ ID NO:7)
	5' AAT GCC GCC GCT TAC TTC CTA- GCG TCT GTC ATG AAA CTG TTT- TCG AT-3' (down) (SEQ ID NO:22)			
pPICZ $\alpha$ A/ apomigren	5' AAG AAT TCC ATC ATC ATC- ATC ACA GCA GC-3' (up) (SEQ ID NO:12)	<i>Eco</i> RI & <i>Not</i> I	Eukaryotic (Yeast), Pichia, pPICZ $\alpha$ A	EFMGSSHHHHHSSGL VPRGSHM- apomigren (SEQ ID NO:23)
	5' AAT GCC GCC GCT TAC TTC CTA- GCG TCT GTC ATG AAA CTG TTT- TCG AT-3' (down) (SEQ ID NO:22)			

Fig. 26C